

SHORT-TERM SCIENTIFIC MISSION, COST863

1) STSM Data.

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Analysis of volatile compounds content in different strawberry varieties

1) Introduction.

Strawberry plant breeding programs in the last 50 years have been focused in the selection of agronomical interesting traits like firmness, production, yield, resistance to diseases, etc (Ulrich et al., 2007). On the contrary, aroma has been pushed into the background in breeding programs, leading to the ‘genetic erosion’ of this trait in strawberry. This fact drives often to the dissatisfaction of the final consumer (Azodanlou et al., 2003). Overall, it is accepted that the more ancient cultivars contain higher aroma values and diversity than the new ones, whereas wild species even overcomes the former group.

In the last years, the study of the diversity of aroma in strawberry is gaining importance regarding its application to breeding programs. Some authors have forced the cross-breeding between wild species and *F. ananassa* cultivars with the purpose of reintroduce the lost aroma diversity in modern cultivars. However, introgression with wild species often results in fertility problems in the progeny due to different ploidy levels (Hanckock and Luby, 1993); therefore, octoploid species such as *F. chiloensis* and *F. virginiana* are specially interesting for the obtaining of improved-aroma seedlings.

Some authors have studied the volatile compounds responsible of the aroma in strawberry fruits. Ulrich et al. (2007) described the main compounds having the strongest impact in aroma by Gas Chromatography – Olfactometry (GCO). In the same work, they described differences in the distribution of key aroma compounds, mainly in esters, terpenes and methyl anthranilate, between some wild strawberry species and the

cultivar 'Elsanta'. The wild *Fragaria* presented higher intensity and diversity of these aroma compounds, specially *F. moschata* which showed the highest values. Aharoni et al. (2004) also reported lower values of terpenes content for cultivated strawberries when compared to *F. vesca* L. We found interesting to perform a similar study in a higher number of accessions grown under southern Europe climate conditions.

2) Objectives.

The collaborative STSM between IFAPA Churriana (Málaga, Spain) and JKI (Quedlinburg, Germany) has pursued, first of all, the knowledge transfer between the centers. The applicant has had the opportunity to learn the backgrounds, technical know-how and processing/interpretation of the data in relation to the analysis of volatile compounds in strawberry. The research group leaded by Dr. Ulrich in the JKI have demonstrated to be in the head of the study of volatile compounds and aroma diversity in strawberry crop. In the other hand, the IFAPA Churriana Center (Málaga, Spain) has an important strawberry genebank of more than 350 entries, counting with cultivars and wild species.

Concerning the research goal of the present STSM, we focused in the non-targeted analysis of volatile compounds distribution, including aromatic and non-aromatic volatiles. Seizing the opportunity of having access to such a high diversity of strawberry accessions, we selected around 50 entries of high interest, including cultivars of american, european and asiatic pedigree, as well as wild species. Our attention focused in the comparison among genotypes in aroma pattern, including qualitative and quantitative aspects. Additionally, some training in the GC/O technique was performed.

3) Material and Methods.

Initially, a total a 42 cultivars and 6 wild species belonging to the IFAPA Churriana genebank collection were selected for harvesting (table 1), although 7 of them were finally discarded due to high variation between samples. Around 200g of fruits were harvested at full-red ripening stage for each genotype in the late crop season, i.e., between June and July 2009. Then, fruits were stored at -20°C until further processing, consisting in homogenization with 200mL of 20% w/v NaCl solution, centrifugation (3000rpm, 20min, 4°C) and recovery of the supernatant, 50mL of which

was stored in brown-coloured bottles together with 12g NaCl. Bottles were stored at -20°C until shipped to JKI Quedlinburg, where instrumental analysis were performed.

Table 1. List of accessions initially selected for the present project.

Cultivars						Wild species
Aromas	Rosana	Diamante	Alta vista	Toyonoka	Coral	<i>F. moschata capron royale</i>
Palomares	Cifrance	Ventana	Festival	Cijosee, Cireine	Macarena	<i>F. vesca reina de los valles</i>
Avalon	Gigantilla	Pedrone	Parker	Everest	Camino Real	<i>F. daltoniana</i>
Galexia	Jucunda	Camarosa	Winterdown	Ruby	Naiad	<i>F. vesca blanca</i>
Captiva	Hummi Gento	Candonga	Commitment	Reusrath aller Krüester	Fuentepina	<i>F. virginiana</i>
Whitney	Premial	Endurance	Virtudes	Hood	Amiga	<i>F. chiloensis</i>
Lanai	Deutche Evern	Honor	Medina	Ville de Paris	Galante	

The volatiles were sampled by head-space solid phase micro-extraction (SPME) following the procedure of Olbricht et al. (2008). Quantitative analysis were carried out with a GC/FID using twofold replication, whereas volatiles compounds were identified in a parallel running in the GC/MS in the electron impact ionization mode (Agilent Technologies Deutschland GmbH, Böblingen, Germany). Gas chromatographic separation in both instruments were performed under identical conditions. Identification of volatile compounds were done using Wiley and NIST libraries.

For data processing, the software CHEMSTATION™ (Agilent) was used in order to determine the number of peaks found in every chromatogram, generating an output of retention times/peak area data pairs. Then, data was further processed with the software CHROMSTAT™, which performed the alignment and pattern recognition of the volatile compounds distribution for all chromatograms. Statistical data analysis, mainly principal component analysis (PCA), was done with STATISTICA.

Finally, some training in the GC/O techniques were performed. Liquid-liquid extraction using dichloromethane was carried out from a mixture of cultivars and wild strawberries in order to ensure the highest diversity of aroma compounds. The procedure was similar to described in Ulrich et al. (2007). Training in the Nasal Impact Factor (NIF) determination of strawberry extractions was also received.

3) Results and discussion.

The chromatograms analysis revealed a total of 194 time intervals (fractions), which putatively correspond with respective volatile compounds appearing at least in one out of the 84 (2x 42 genotype) chromatograms of the analysis set. The sum of peaks areas are averaged in table 2 for every genotype.

Table 2. Mean, standard deviation and coefficient of variation for the sum of the peaks areas found in every genotype.

	Genotype	Relative		
		concentration	± s.d.	c.v.
Wild	F. Virginiana	25472	87	0.3
	F. Vesca Reina de los Valles	13970	830	5.9
	F. Vesca Blanca	13273	294	2.2
	F. Moschata (Capron Royale)	11051	462	4.2
	Total	16597	5637	
Cultivars	Toyonoka	9572	126	1.3
	Fuentepina	5990	41	0.7
	Avalon	4463	230	5.1
	Winterdown	4290	167	3.9
	Diamante	4200	34	0.8
	Coral (2)	3766	96	2.5
	Endurance	3558	102	2.9
	Camino Real	3524	27	0.8
	Hummi Gento	3415	78	2.3
	Pedrone	3229	13	0.4
	Ville de Paris	3201	22	0.7
	Naiad	3057	11	0.4
	Reusrath aller Krüester	3055	23	0.8
	Palomares	2883	98	3.4
	Camarosa	2774	163	5.9
	Commitment	2589	4	0.1
	Macarena	2524	329	13.1
	Honor	2462	136	5.5
	Virtudes	2428	110	4.5
	Ruby	2423	20	0.8
	Everest	2412	32	1.3
	Cifrance	2370	38	1.6
	Deutsche Evern	2036	65	3.2
	Aromas	2015	14	0.7
	Premial	1999	31	1.6
	Jucunda	1986	71	3.6
	Hood	1958	53	2.7
	Ventana	1935	23	1.2
	Medina	1708	33	1.9
	Rosana	1707	11	0.7
	Cijosee, Cireine	1706	23	1.3
	Galexia	1512	149	9.9
	Lanai	1503	111	7.4
Galante	1369	7	0.5	
Amiga	1079	8	0.8	
Festival	1052	3	0.3	
Gigantilla	897	29	3.2	
Captiva	697	16	2.3	
	Total	3031	1644	

Overall values for wild strawberries were higher than cultivars, being 16597 ± 5637 and 3031 ± 1644 , respectively. This result denotes higher aroma intensities for the wild genotypes, accordingly to previous works (Ulrich et al., 2007). Within the wild strawberry group, *F. virginiana* presented the highest values (26217 ± 91), whereas *F. moschata capron royale* showed the lowest levels (11051 ± 462). In turn, maximum value for cultivars went to Toyonoka (9572 ± 126), whereas Captiva showed the poorest levels (679 ± 16). Interestingly, Fuentepina, a brand new variety from the Spanish National Breeding Program, showed the second highest levels of total peak areas in cultivars (5990 ± 41).

Out of the 194 fractions, a total of 51 compounds were successfully identified by GC-MS library data, from which 20 were additionally identified using external references (table 3). Only two fractions were found that contained more than one compound (i.e., they were not separated under the assayed conditions (Id. no. 38 and 95 in table 3)), whereas in two other fractions it was unable to distinguish between isomers (Id. no. 42 and 44, table 3).

Table 3. Results of relative concentrations and percentage of total peak area for the identified compounds.

Volatile compounds	ID no.	Relative concentration means			% of total peak area				
		Cultivars	Wild	Max	Cultivars	Wild	Max		
Esters	Ethyl acetate	3	1.4	119.7*	385.4	0.1	0.7	2.6	
	Methyl butanoate	6	108.7*	26.0	406.8	6.1	0.2	23.6	
	Ethyl butanoate	11	60.5	1227.1*	4002.4	2.7	7.4	16.6	
	Buthyl acetate	17	6.3	85.1*	176.9	0.3	0.6	2.8	
	Methyl hexanoate	32	147.2	161.2	1622.2	5.9	1.2	19.2	
	Buthyl butanoate + (E) 2-Hexenal	38	14.3	49.3*	103.7	0.7	0.4	5.4	
	Ethyl Hexanoate	40	133.4	2155.0*	6711.4	4.9	12.8	27.9	
	Hexyl acetate	45	124.1	616.0*	777.5	7.0	5.0	17.7	
	(Z) 3-hexen 1-ol acetate	51	0.0	51.1*	166.8	0.0	0.5	1.8	
	(E) 2-Hexenyl acetate	53	264.8	485.4*	992.9	14.4	4.3	40.5	
	2-hexenoic acid ethylester	56	0.0	10.3*	46.2	0.0	0.0	0.2	
	Butanoic acid hexylester	65	1.3	14.6	29.3	0.1	0.1	0.8	
	Acetic acid octylester	74	10.5	460.4*	913.1	0.5	3.4	4.5	
	Acetic acid decylester	109	3.3	87.6*	134.5	0.2	0.7	1.1	
	Acetic acid phenil methyl ester	120	24.2	28.4	89.2	1.5	0.2	5.9	
	Methyl anthranilate	181	0.0	86.4*	185.2	0.0	0.9	1.9	
	Cinnamyl acetate	175	0.0	260.5*	430.9	0.0	2.5	4.6	
	Aldehydes	Hexanal	18	2.3	22.1*	47.5	0.1	0.2	0.9
		2-pentanone	5	0.0	323.5*	614.2	0.0	2.4	3.6
Ketons	2-heptanone	31	10.8	2024.9*	3611.5	0.5	16.1	28.2	
	2-nonanone	60	10.9	2539.5*	3843.1	0.5	19.1	32.2	
	2-tridecanone	132	0.5	115.7*	373.8	0.0	1.1	3.9	
	β-Damascenone	135	6.1	61.2*	85.7	0.4	0.5	1.6	
	2-heptanol	52	2.0	3.9	19.7	0.1	0.0	1.2	
Alcohols	2-methyl 6-hepten 1-ol	73	11.0*	0.0	41.1	0.7	0.0	3.8	
	2-ethyl 1-hexanol	76	9.6	17.5	43.7	0.7	0.1	2.1	
	Nonanol	79	2.1	446.1*	904.0	0.1	3.3	7.6	
	1-octanol	85	5.9	356.9*	625.8	0.3	2.6	4.7	
	Undecanol	117	0.0	30.9*	47.7	0.0	0.2	0.4	
Acids	2-methyl butanoic acid	107	20.7	20.0	136.2	1.1	0.2	4.0	
	Hexanoic acid	138	40.3	140.9*	356.1	1.9	1.1	4.8	
Terpenes	Limonene	34	39.9	27.7	107.6	2.4	0.2	6.1	
	γ- terpinen or z-cymen	42	25.4	69.6	107.5	1.5	0.6	10.2	
	o-cymen or para-cymen	44	2.8	79.1*	153.7	0.1	0.6	1.3	
	Terpinolen	46	57.0	32.5	275.9	2.8	0.3	12.7	
	Linalool	84	250.6*	75.6	1815.0	9.8	0.6	40.0	
	Terpinen 4-ol + undecanon	95	58.4	129.5*	207.4	3.9	1.2	15.9	
	Myrtenal	100	2.9	116.4*	176.8	0.4	0.9	4.8	
	β-farnesene	106	0.0	29.5	48.3	0.0	0.2	4.1	
	Myrtenyl acetate	110	6.9	204.5*	306.6	0.5	1.8	2.6	
	α-terpineol	112	162.2*	46.6	737.9	8.5	0.4	20.1	
	β-citronellol	124	0.0	33.5	57.2	0.0	0.2	0.4	
	Myrthenol	130	2.8	0.0	28.7	0.1	0.0	0.8	
	Geraniol	140	9.5	57.7	90.9	0.6	0.5	2.0	
	Nerolidol	163	115.5*	46.4	757.4	5.7	0.5	26.4	
Lactones	Bisabolool oxid B	173	72.4*	0.0	223.8	4.7	0.0	27.9	
	Decalactone	174	34.7	19.2	310.4	1.6	0.2	14.6	
	Undecalactone	189	13.1*	0.0	63.0	0.7	0.0	2.8	
Furans	Methoxyfuraneol	92	35.8	216.8*	735.5	2.9	1.3	31.0	
Miscellaneous	Methoxy phenyl oxyme	121	10.6*	0.0	54.8	0.8	0.0	4.9	
	Eugenol	176	4.3	58.9*	103.8	0.2	0.6	1.2	

Bold letters are compounds identified by external references; normal letters by GCMS library search. Asterisks indicate statistical differences between cultivars and wild species (P=0.05).

Within the wild strawberries, the two major components found belong to the ketone group: 2-nonanone and 2-heptanone, representing 19 % and 16 %, respectively. Esters were also importantly represented, mainly by ethyl hexanoate (13 %) and butyl acetate (7 %). In the group of cultivars, the ester compound (E)-2-hexenyl acetate presented the highest values (14 %), followed by terpenes (mainly linalool, 10 % and α -terpineol, 9 %). Nevertheless, the higher relative amount of these volatile compounds does not imply necessarily the higher importance or contribution to the overall aroma-sensory impression. Rather, the odor activity value (OAV) of a certain compound is the appropriate parameter dealing with this issue, and depends on its concentration and threshold in air (Schieberle et al., 1997).

Comparing the raw data averages of these 54 compounds in cultivars and wild genotypes, we established 4 different groups attending to their presence/absence or significantly higher amount in each genotype group (table 4).

Table 4. Qualitative comparison of volatile compounds between wild strawberries and cultivars.

Exclusive for wild	Undecanon ¹	Exclusive for cultivars	Diethoxy phenyl oxyme
	Methyl anthranilate ¹		Myrthenol
	2-Pentanone		Bisabolool oxid
	(Z)-3-hexen 1-ol acetate		
	Nonanal		
	Dodecanol		
	β -citronellool		
	Cinamyl acetate		
Higher in wild	Ethyl butanoate ¹	Higher in cultivars	Methyl Butanoate ¹
	Buthyl butanoate ¹		Linalool ¹
	Ethyl Hexanoate ¹		Undecalactone ¹
	O-cymene ¹		Limonen
	Hexyl acetate ¹		Terpinolen
	(E)-2-Hexenyl acetate		2-methyl 6-hepten 1-ol
	Myrtenyl Acetate ¹		α -terpineol
	β -Damascenone ¹		Nerolidol ¹
	Hexanoic acid ¹		
	Ethyl Acetate		
	Buthyl Acetate		
	Hexanal		
	2-heptanone		
	2-Nonanone		
	Acetic acid octylester		
	Nonanol		
	1-octanol		
	Myrtenal		
	Acetic acid decylester		
	2-tridecanon		
Geraniol			
Eugenol			
Terpinen 4-ol			

Some of the above volatiles have been described as detectable in GCO analysis and therefore as important contributors in strawberry aroma (Ulrich et al., 2007), e.g., some of the esters (methyl and ethyl butanoate, butyl acetate, ethyl hexanoate, hexyl acetate), terpinen-4-ol, hexanoic acid, eugenol and methyl anthranilate. All of them are found in higher levels in wild species, except methyl butanoate. Surprisingly, in this study we found 3 volatiles that are exclusive for cultivars (table 4), although none of them is considered as a key compound for aroma trait.

The principal component analysis (PCA) of the 51 volatiles identified in the 42 genotypes was done (figure 1). Two main clusters were observed clearly separating the 4 wild species and the 38 cultivars analyzed. *Fragaria virginiana* (no. 432) is the genotype that further moved away from the rest, denoting the most different volatiles pattern compared to the rest of accessions. In turn, three of the cultivars also presented differences in volatiles distribution: Toyonoka (232), Fuentepina (412) and Winterdown (52).

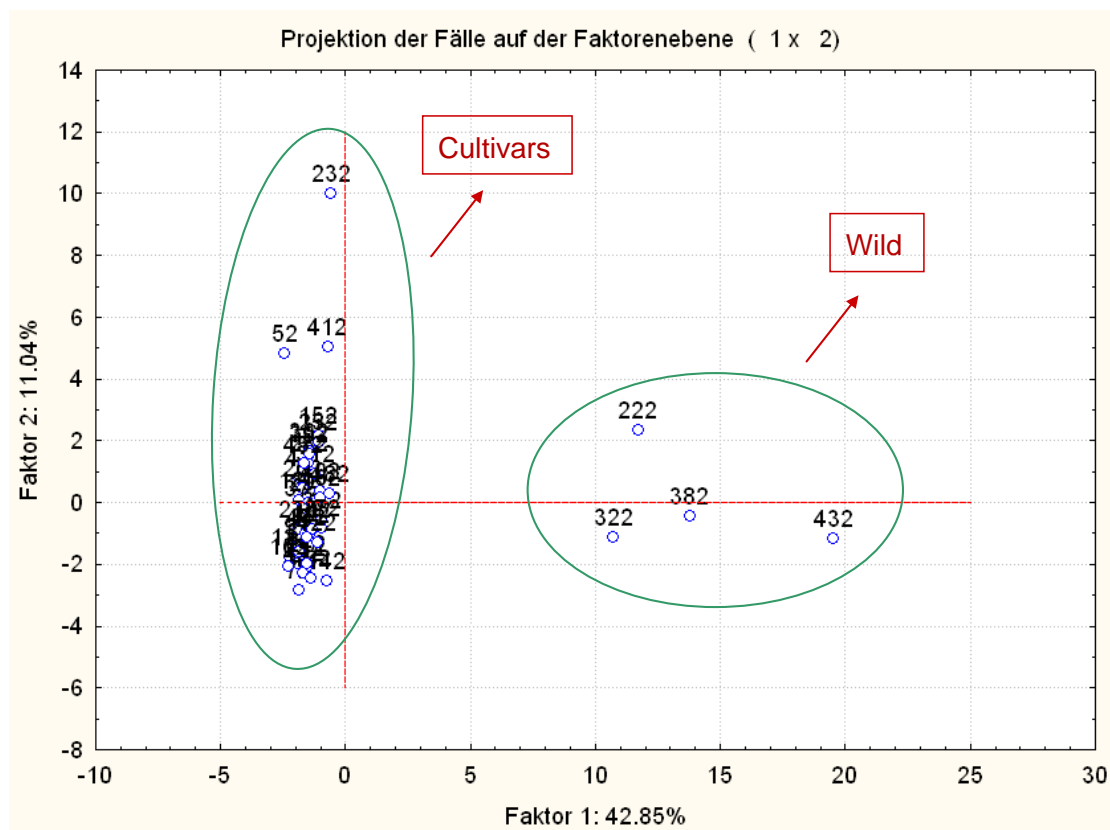


Figure 1. PCA of a family of 51 volatile compounds present in 42 genotypes, including cultivars and wild strawberries.

Further PCA analysis done exclusively with cultivars (i.e., removing wild species data) confirmed the former trend (figure 2). Toyonoka, Fuentepina and Winterdown clearly split off from the main cluster, the first cultivar presenting the higher differences with respect to the rest. As mentioned above, Toyonoka and Fuentepina also exhibited the highest levels of total peaks areas. Therefore, these two cultivars showed differences both in the quantitative level and in volatiles distribution.

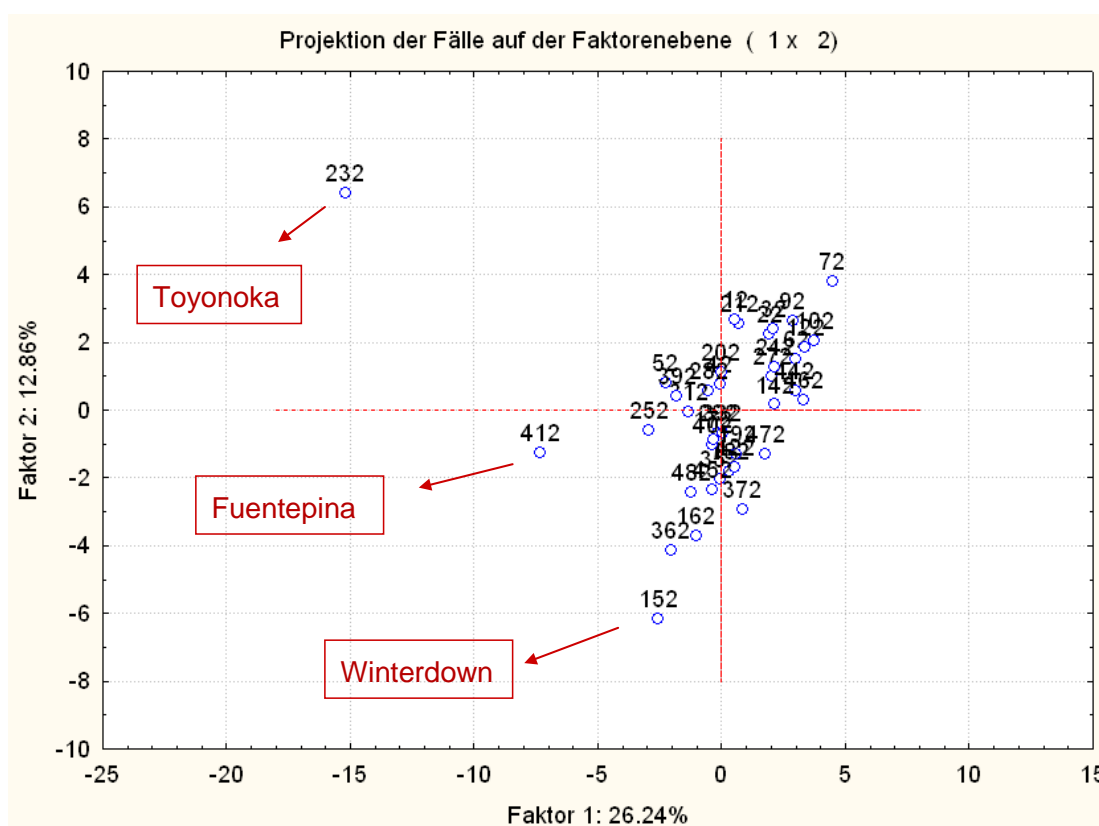


Figure 2. PCA of a family of 51 volatile compounds present in 38 cultivars.

4) Future perspectives.

Currently come of the data of the present project are still under statistical processing, and some results are being written for a possible publication in a peer-reviewed journal and for the next IHC Berries Symposium (Lisbon, 2010). Additionally, the guest of the present STSM has recently applied for a postdoctoral grant from the Spanish Government (Ministerio de Educación) to be held in the JKI Quedlinburg (Germany) under the supervision of Dr. Ulrich.

5) Acknowledgements.

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